

Appendix

Clones bd107_16, bm41_7, br342_11, ej258_11, k232_2x, lf307_5 and lr204_1 have been deposited with the American Type Culture Collection in this composite deposit, from which each clone comprising a particular polynucleotide is obtainable. Each clone has been transfected into separate bacterial cells (*E. coli*) in this composite deposit. The deposited cDNA can be removed from its vector by digestion with restriction enzymes (5' site, EcoRI; 3' site NotI). Bacterial cells containing a particular clone can be obtained from the composite deposit as follows:

An oligonucleotide probe should be designed to the sequence that is known for that particular clone. This sequence can be derived from the sequences provided in the attached Sequence Listing, or preferably, from a composite of those sequences. The sequence of an oligonucleotide probe that was used to isolate or to sequence each full-length clone is identified below, and should be most reliable in isolating the clone of interest.

Clone	Probe Sequence ¹
bd107_16	SEQ ID NO:198
bm41_7	SEQ ID NO:199
br342_11	SEQ ID NO:200
ej258_11	SEQ ID NO:201
k232_2x	SEQ ID NO:202
lf307_5	SEQ ID NO:203
lr204_1	SEQ ID NO:204

The following SEQ ID NO:s in the attached Sequence Listing correspond to the indicated clone: bd107_16: SEQ ID NO:183 ; bm41_7: SEQ ID NO:185 ; br342_11: SEQ ID NO:187 ; ej258_11: SEQ ID NO:189 ; k232_2x: SEQ ID NO:191 ; lf307_5: SEQ ID NO:193, SEQ ID NO:195 ; lr204_1: SEQ ID NO:196 ;

In the sequences listed above which include an N at position 2, that position is occupied in preferred probes/primers by a biotinylated phosphoramidite residue rather than a nucleotide (such as , for example, that produced by use of biotin phosphoramidite (1-dimethoxytrityloxy-2-(N-biotinyl-4-aminobutyl)-propyl-3-O-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite) (Glen Research, cat. no. 10-1953)).

¹ References are to sequences in the Sequence Listing attached hereto.

The design of the oligonucleotide probe should preferably follow these parameters:

- (a) It should be designed to an area of the sequence which has the fewest ambiguous bases ("N's"), if any;
- (b) It should be designed to have a T_m of approx. 80 ° C (assuming 2° for each A or T and 4 degrees for each G or C).

The oligonucleotide should preferably be labelled with γ - ^{32}P ATP (specific activity 6000 Ci/mmol) and T4 polynucleotide kinase using commonly employed techniques for labelling oligonucleotides. Other labelling techniques can also be used. Unincorporated label should preferably be removed by gel filtration chromatography or other established methods. The amount of radioactivity incorporated into the probe should be quantitated by measurement in a scintillation counter. Preferably, specific activity of the resulting probe should be approximately 4×10^6 dpm/pmol.

The bacterial culture containing the pool of full-length clones should preferably be thawed and 100 μl of the stock used to inoculate a sterile culture flask containing 25 ml of sterile L-broth containing ampicillin at 100 $\mu\text{g}/\text{ml}$. The culture should preferably be grown to saturation at 37°C, and the saturated culture should preferably be diluted in fresh L-broth. Aliquots of these dilutions should preferably be plated to determine the dilution and volume which will yield approximately 5000 distinct and well-separated colonies on solid bacteriological media containing L-broth containing ampicillin at 100 $\mu\text{g}/\text{ml}$ and agar at 1.5% in a 150 mm petri dish when grown overnight at 37°C. Other known methods of obtaining distinct, well-separated colonies can also be employed.

Standard colony hybridization procedures should then be used to transfer the colonies to nitrocellulose filters and lyse, denature and bake them.

The filter is then preferably incubated at 65°C for 1 hour with gentle agitation in 6X SSC (20X stock is 175.3 g NaCl/liter, 88.2 g Na citrate/liter, adjusted to pH 7.0 with NaOH) containing 0.5% SDS, 100 $\mu\text{g}/\text{ml}$ of yeast RNA, and 10 mM EDTA (approximately 10 mL per 150 mm filter). Preferably, the probe is then added to the hybridization mix at a concentration greater than or equal to 1×10^6 dpm/mL. The filter is then preferably incubated at 65°C with gentle agitation overnight. The filter is then preferably washed in 500 mL of 2X SSC/0.5% SDS at room temperature without agitation, preferably followed by 500 mL of 2X SSC/0.1% SDS at room temperature with gentle shaking for 15 minutes. A third wash with 0.1X SSC/0.5% SDS at 65°C for

30 minutes to 1 hour is optional. The filter is then preferably dried and subjected to autoradiography for sufficient time to visualize the positives on the X-ray film. Other known hybridization methods can also be employed.

- 5 The positive colonies are picked, grown in culture, and plasmid DNA isolated using standard procedures. The clones can then be verified by restriction analysis, hybridization analysis, or DNA sequencing.

10 Once a particular clone has been isolated from the composite deposit, the cDNA can be removed from the vector in which it was deposited by performing an EcoRI/NotI digestion (5' site, EcoRI; 3' site, NotI). The resultant EcoRI/NotI fragments should have the following approximate sizes:

	<u>Clone</u>	<u>Size</u>
15	bd107_16	1500 bp
	bm41_7	1700 bp
	br342_11	1400 bp
	ej258_11	670 bp
	k232_2x	555 bp
20	lf307_5	1000 bp
	lr204_1	900 bp

Clone Origin

- 25 All clones in the deposit are of human origin.